

Attorney Docket No.: **RU-0224**  
Inventors: **Fennell and Haggblom**  
Serial No.: **10/828,781**  
Filing Date: **April 21, 2004**  
Page 5

**REMARKS**

Claims 1-7 are pending in this application. Claims 1-7 have been rejected. Claims 2 and 6 have been canceled. Claims 1 and 5 have been amended. No new matter has been added. Reconsideration is respectfully requested in view of the amendments to the claims and following remarks.

**I. Rejection of the Claims Under 35 U.S.C. §103**

Claims 1-7 remain rejected under 35 U.S.C. 103(a) as being unpatentable over Adrian et al. taken with Maymo-Gatell et al. in light of Bunge et al. Based upon the teachings of the cited references, the Examiner concludes that it would have been *prima facie* obvious for one of ordinary skill in the art to modify the process of Adrian et al. by replacing strain *Dehalococcoides* CBDB1 with strain *Dehalococcoides ethenogenes* 195 in view of their close relatedness and the teachings of Maymo-Gatell et al. regarding the dehalogenating properties of strain 195 for the expected benefit of providing an effective process of bioremediation for very toxic and health-damaging environmental pollutants, such as dioxins. The Examiner further suggests that Applicants arguments concerning the prior art are not persuasive since obviousness does not require absolute predictability only a reasonable expectation of success.

Applicants respectfully disagree with this conclusion and traverse this rejection. As discussed in detail in the previous reply dated March 12, 2009, Based upon the 16S rRNA gene sequences, Adrian et al. classify CBDB1 and *Dehalococcoides ethenogenes* as distinct organisms. See Figure 2, which depicts the phylogenetic relationship of CBDB1 and *D. ethenogenes*. In

Attorney Docket No.: **RU-0224**  
Inventors: **Fennell and Haggblom**  
Serial No.: **10/828,781**  
Filing Date: **April 21, 2004**  
Page 6

this respect, Bunge et al. indicate that "*Dehalococcoides* sp. strain CBDB1 (ref 3.) is the only known bacterium able to dechlorinate chlorinated benzenes, and *D. ethenogenes* strain 195 completely dechlorinates tetrachloroethene to ethene." See page 358, ¶2. While both strain CBDB1 and strain 195 use reductive dehalogenation for energy conservation and growth, Adrian et al. particularly point out that these two organisms have distinct metabolic requirements; the cultivation of *D. ethenogenes* requires the addition of undefined supplements, whereas the cultivation of strain CBDB1 is achieved on entirely synthetic medium. See abstract and second full paragraph, column 1, page 583. Thus, based upon the combined teachings of Adrian et al. and Bunge et al., the skilled artisan would conclude that there are clear metabolic differences between CBDB1 and *D. ethenogenes* that can not be predicted from 16S rRNA sequences. In fact, it has been demonstrated that population analysis of *Dehalococcoides* spp. using the 16S rRNA gene fails to differentiate strains because strains with the same 16S rRNA gene sequence can have different dehalogenating abilities. See abstract of Cupples (2008) *J Microbiol Methods.* 72(1):1-11; enclosed herewith.

Applicants respectfully submit that while Adrian et al. may teach dehalogenation of aromatic chlorinated compounds with *Dehalococcoides* CBDB1, Maymo-Gatell et al. may teach that strain 195 can dehalogenate tetrachloroethene, and Bunge et al. may teach a similarity between the 16S rRNA sequence of CBDB1 and strain 195, the prior art would suggest, as evidenced by Ward and Cupples, that relatedness of 16S rRNA sequences is not predictive of the functional genes an organism may possess. Indeed, there is no evidence of record to suggest that strain 195 has the same

Attorney Docket No.: **RU-0224**  
Inventors: **Fennell and Haggblom**  
Serial No.: **10/828,781**  
Filing Date: **April 21, 2004**  
Page 7

metabolic properties of CBDB1. In fact, the cultivation requirements of these strains, as disclosed by Adrian et al., would indicate that strain 195 and CBDB1 have distinct substrate utilization profiles. Therefore, Applicants submit that the combination of prior art would not provide one of skill with a reasonable expectation of success based on the lack of predictability.

However, in an earnest effort to advance the prosecution and facilitate allowance of the claims, Applicants have amended the claims to recite that the methods of the present invention involve use of a mixed culture of bacteria, wherein the mixed culture contains *Dehalococcoides ethenogenes* strain 195 and another *Dehalococcoides ethogenes* strain, and wherein the *Dehalococcoides ethenogenes* strain 195 is present in the culture at a concentration percentage of from 30 to 80 percent of the total culture. Support for these amendments to the claims can be found throughout the specification as filed but in particular at pages 16-17, including a teaching of the concentration percentage range of the bacteria *Dehalococcoides ethenogenes* strain 195 at page 17, lines 14-18.

MPEP 2143 states that in order to establish a *prima facie* case of obviousness the references cited and combined must teach the limitations of the claims. As discussed *supra*, the cited references fail to teach the limitations of the claim as amended which recites that the methods of the present invention involve use of a mixed culture of bacteria, wherein the mixed culture contains *Dehalococcoides ethenogenes* strain 195 and another *Dehalococcoides ethogenes* strain, and wherein the *Dehalococcoides ethenogenes* strain 195 is present in the culture at a

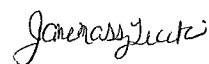
Attorney Docket No.: **RU-0224**  
Inventors: **Fennell and Haggblom**  
Serial No.: **10/828,781**  
Filing Date: **April 21, 2004**  
Page 8

concentration percentage of from 30 to 80 percent of the total culture. Accordingly, the references, either when combined or alone fail to teach or suggest the limitations of the claim as amended and withdrawal of this rejection is respectfully requested.

## **II. Conclusion**

Applicants believe that the foregoing comprises a full and complete response to the Office Action of record. Accordingly, favorable reconsideration and subsequent allowance of the pending claims is earnestly solicited.

Respectfully submitted,



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Date: **August 25, 2009**

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